植物研究雑誌
J. Jpn. Bot.
74: 84–95 (1999)

Embryological Studies in *Euchresta japonica* (Leguminosae)

Hiroyoshi OHASHI, Kazue TANAKA and Tomoyuki NEMOTO

Biological Institute, Graduate School of Science, Tohoku University, Sendai, 980-8578 JAPAN

(Received on July 15, 1998)

Embryological features in *Euchresta japonica*, a species of the monotypic tribe Euchresteae, are described for the first time. Features in microsporogenesis are as follows: the anthers are tetrasporangiate; anther wall development corresponds to the dicotyledonous type; the tapetum is of the glandular type and uninucleate; the microspore tetrad is tetrahedral. Pollen grains are 2-celled at the time of shedding. These features are common with those generally found in Papilionoideae to date. Features in megasporogenesis are as follows: the archesporium is one-celled and hypodermal; the ovule is bitegmic, campylotropous and crassinucellate; one megaspore mother cell forms a linear tetrad. Megagametogenesis is of the Polygonum type; the antipodals are very ephemeral and most of the polar nuclei are in contact with each other and fused before fertilization. These embryological features of *Euchresta* show that the tribe Euchresteae is distinct from related tribes Sophoreae and Thermopsideae and may suggest that it is an advanced tribe related to *Sophora* and *Maackia*.

Key words: Embryology, *Euchresta*, Leguminosae, *Sophora*, Thermopsideae

Introduction

Euchresteae (Leguminosae-Papilionoideae) is a monotypic tribe based on the genus *Euchresta*. It was established by Ohashi in 1973. *Euchresta* is comprised of five species and is endemic to subtropical to warm temperate regions of eastern and southeastern Asia. The species are evergreen subshrubs or small shrubs up to 2 m high and have blackish purple ellipsoidal fleshy fruits. The fruit is covered with a membranous pericarp and contains one seed. There has been no agreement on the systematic relation of *Euchresta* to other genera (Bentham 1860, Ohashi and Sohma 1970, Polhill 1981, Chen et al. 1992, Doyle et al. 1997).

In order to clarify the systematic position of tribe Euchresteae, its embryological features, especially microsporogenesis, microgametogenesis, megasporogenesis, and megagametogenesis, were examined. Embryological studies are often valuable in estimating relationships among taxa in higher rank above species even when their mature morphological features are remarkably different from each other, because they offer many comparable characteristics among those taxa at every developmental stage (e.g., Maheshwari 1963, Maheshwari and Kapil 1966, Palser 1975, Herr 1984, Tobe 1989). No embryological studies, however, have been made on Euchresteae. Prakash (1987) pointed out that the tribe is the one in need of embryological study in order to review legume embryology on the whole.

In the present study, embryological features of *Euchresta japonica* are compared with those of some species belonging to Sophoreae and Thermopsideae. Sophoreae (Ohashi and Sohma 1970, Polhill 1981, Chen et al. 1992, Doyle et al. 1997) and Thermopsideae (Chen et al. 1992, Doyle et al. 1997) are regarded as close to Euchresteae. Dalbergieae was also considered to be related to Euchresteae (Bentham 1860, Bentham and Hooker 1865, Hutchinson 1964), but was regarded to be not related (Ohashi 1973, Polhill 1981). This view was supported by Doyle et al. (1997), and, hence, Dalbergieae was not treated in this work.

Materials and Methods

Buds and flowers of *Euchresta japonica* Hook.f. ex Regel were collected at consecutive stages of development and fixed in FAA at Aki-shi in Kouchi Prefecture, Japan in 1995. The following materials were also collected in 1995, mainly at the mature flowering stage: *Cladrastis sikokiana* (Makino) Makino (Sophoreae) wild in Ueno-mura in Gunma Prefecture, Japan, *Sophora tomentosa* L. (Sophoreae) wild in Chichi-jima, Ogasawara-mura in Tokyo Prefecture, Japan, and *Styphnolobium japonicum* (L.) Schott, *Sophora flavescens* Ait., *Maackia amurensis* Rupr. & Maxim. subsp. *buergeri* (Maxim.) Kitam. (all Sophoreae) and *Thermopsis lupinoides* (L.) Link (Thermopsideae) cultivated in Sendai-shi in Miyagi Prefecture, Japan. The voucher specimens are in TUS.

All materials were dehydrated through a *t*-butyl alcohol series, embedded in paraffin, and sectioned at a thickness of 6–8 μm with a rotary microtome. The sections were stained with iron hematoxylin and fast-green (Sass 1958) and mounted with Entellan. A total of 175 ovules from 10 individuals of *E. japonica* at various stages of development, 240 from two individuals of *C. sikokiana*, 104 from one individual of *Sty. japonicum*, 81 from one individual of *Sop. tomentosa*, 147 from one individual of *Sop. flavescens*, 69 from one individual of *M. amurensis*, and 155 from eight individuals of *T. lupinoides* were observed with a light microscope.

Observation

1. Anther, microsporogenesis, and male gametophyte of *Euchresta japonica*

The anther is tetrasporangiate and its wall consists of four cell layers, the epidermis, endothecium, middle layer and tapetum (Fig. 1). This is referable to the dicotyledonous type (Davis 1966). During anther development, the middle layer is divided periclinally into two or three layers (Fig. 2). The tapetum consists of a single glandular layer and its cells are uninucleate (Fig. 3). When the pollen grains are mature the middle layers degenerate and the tapetum disappears (Fig. 4). The endothecial cells are irregular in shape (Fig. 5). The septum of the anther breaks down and shrinks, and the anther becomes bilocular. At anthesis the anther dehisces by two longitudinal slits (Fig. 6).

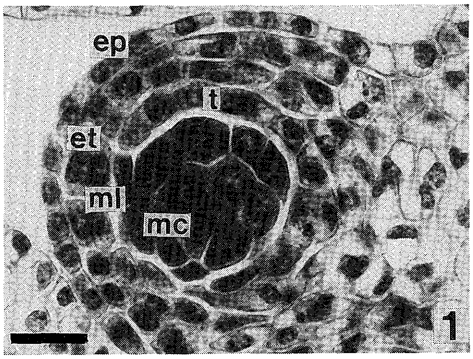
Microspore mother cells are 10–15 μm in diameter and are easily distinguishable from the parietal cells by their larger size, more darkly stained cytoplasm, and larger nucleus (Figs. 1, 2). The microspore mother cells undergo meiosis (Fig. 7) and cytokinesis simultaneously resulting in tetrahedral tetrads (Fig. 8). Each tetrad is enveloped by a callose membrane (Fig. 3), and then the microspores are separated each other (Fig. 4).

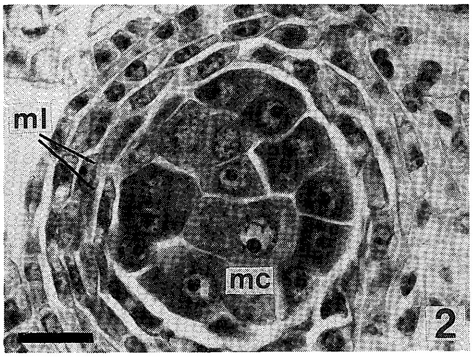
Mature pollen grains are two-celled (Fig. 9). One of them has a smaller generative nucleus and the other has a larger vegetative nucleus.

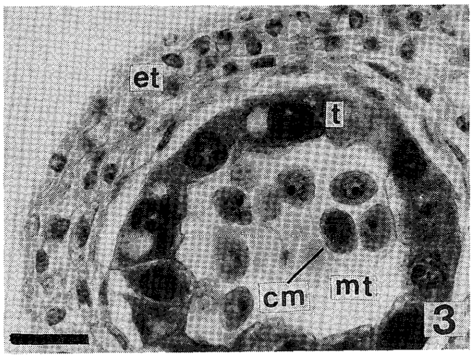
2. Morphology of the ovary and ovule of *Euchresta japonica*

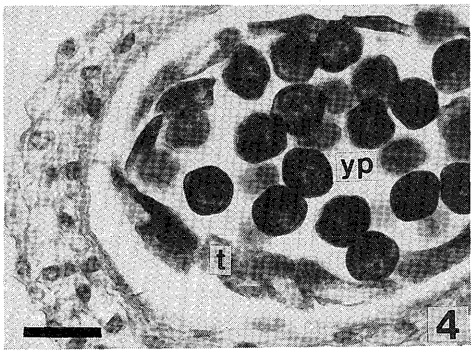
The ovary is monocarpellate, uniloculate, and it contains usually one, sometimes two ovules on the marginal placenta. Among 147 flowers examined, 101 flowers had one ovule and 36 flowers had two ovules, while 10 flowers had no ovules.

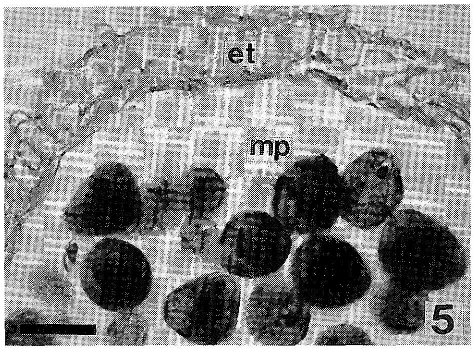
The mature ovule is campylotropous and about 0.5 mm wide (Fig. 19). The ovule is bitegmic and curved toward the apex. The outer integument consists of seven to ten layers of cells of which the size and shape are

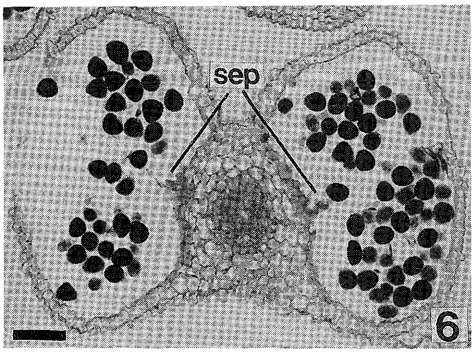


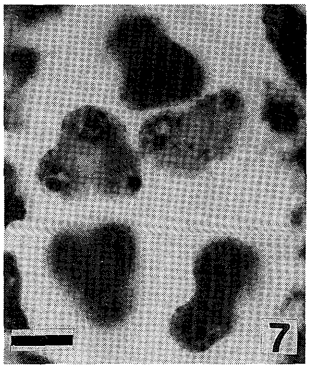


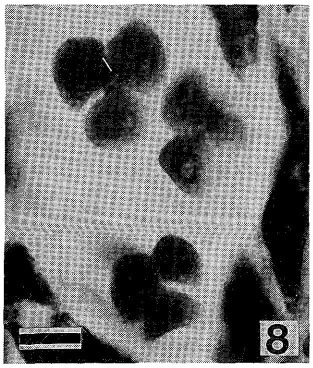


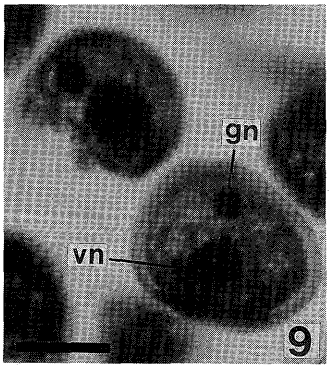












almost uniform. The inner integument consists of two uniform layers of cells. The micropylar end of the integuments becomes thicker than other parts. The outer integument becomes 10–13 layers of cells, while the inner integument increases to 5–6 layers.

Vessel elements with a spiral thickening were observed from the funicular to the chalazal end of the outer integument.

3. Megasporogenesis of *Euchresta japonica*

The relationship between flower size and the number of ovules observed in our study is shown in Table 1 as indicating the stage of megasporogenesis. Megaspore mother cells were observed in flower buds of 3.5 to 4.5 mm long. The embryo sac including eight nuclei, which represents the Polygonum type arrangement, was actually observed in those of 5.5–5.7 mm long. Mature flowers with no antipodals were about 12 mm long. Some ovules were nutrient or abnormal, in which the number of nuclei and their distribution pattern are different from normal ones.

At the initiation of integuments an archesporial cell having a large nucleus appears under the nucellar epidermis. It divides periclinally resulting in a parietal cell and a sporogenous cell (Fig. 10). Thus, the ovule is crassinucellate. In most cases a sporogenous cell is differentiated into one megaspore mother cell (Fig. 11), or rarely into two megaspore mother cells.

The megaspore mother cell is seven to eight times as large as the surrounding cells, so it is easily recognizable. It elongates (Fig. 12) and undergoes meiosis resulting in a linear megaspore tetrad (Figs. 13, 14). Those three nearest the micropyle degenerate, and the only one nearest the chalazal end becomes larger and functional. Meiosis seems to progress rapidly because only three ovules out of 29 in the same size class contain megaspore tetrads though many megasporocytes and functional megaspores are observed (Table 1).

4. Embryo sac formation of *Euchresta japonica*

The larger megaspore undergoes three nuclear divisions (Figs. 15, 16) resulting in an eight-nucleate and seven-celled embryo sac of the Polygonum type. Antipodals are never observed in the mature embryo sac (Figs. 17, 18, Table 1) and they are considered to be very ephemeral. No starch grains are observed in the embryo sac.

The mature embryo sac elongates toward the chalazal end and its chalazal side reaches the bottom of the inner integument. The nucellar tissue which is adjacent to the embryo sac is vacuous. Two polar nuclei come into contact with each other (Fig. 18), and in most cases they fuse resulting in one large nucleus before fertilization.

5. Comparison of ovular and embryo sac features among the species examined

The mature ovule and embryo sac of *Euchresta japonica* are compared with those of two species of *Sophora* and one species each of *Styphnolobium*, *Cladrastis*, *Maackia* and *Thermopsis*, respectively (Fig. 19, Table 2).

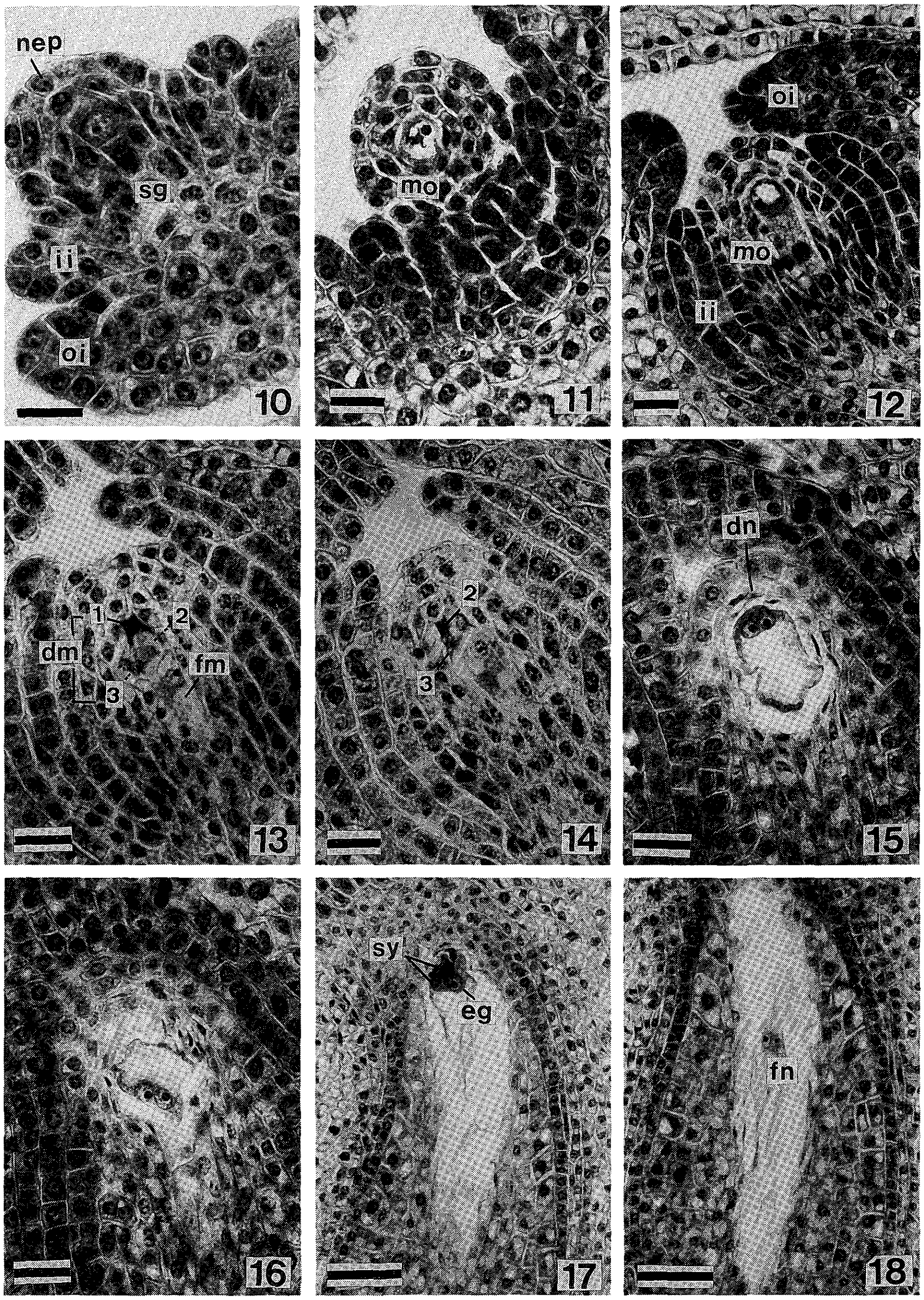
Ovules of *E. japonica* and *T. lupinoides* are larger and more strongly curved than those of other species. The ovule of *E. japonica* is

Figs. 1–9. Anther wall development and microsporogenesis of *Euchresta japonica*. 1. Locule of a very young anther at sporogenous stage. 2. Locule of an anther at sporogenous stage. 3. Anther wall at pollen tetrad stage. Tetrads are enveloped by callose membrane. 4. Degenerating tapetum and young pollen grains. 5. Mature anther wall and 2-celled mature pollen grains. 6. Anther at anthesis with 2 locules. 7. Meiosis in microsporocyte. 8. Cytokinesis in meiosis. 9. 2-celled mature pollen grain. Abbreviations: cm, callose membrane; ep, epidermis; et, endothecium; gn, generative nucleus; mc, microsporocyte; ml, middle layer; mp, mature pollen grains; mt, microspore tetrad; sep, degenerating septum; t, tapetum; vn, vegetative nucleus; yp, young pollen grains. Bars = 20 μm (1–5), 50 μm (6), and 10 μm (7–9).

Table 1. Number of ovules in relation to flower size and developmental stages of megagametophyte in *Euchresta japonica*

Flower size (cm)	–2.5	2.5–3.5	3.5–4.5	4.5–5.5	5.5–6.5	6.5–7.5	7.5–8.5	8.5–9.5	9.5–10.5	10.5–12	
Developmental stages											Total
archesporial cell	12	2									14
sporogenous cell		9									9
megasporocyte		8	18	1							27
elongated m.c.			2								2
megaspore tetrad			3								3
1 nucleus				1				1		2	4
2 nuclei			1		1	1				1	4
4 nuclei			2	4	3				2	1	12
8 nuclei				1							1
3+2+3				1	5	1	1				8
3+2+X				3	5	2	1	1		3	15
3+2+0				1	6	4		1	4	13	29
3+1+X								1			1
3+1+0							2	2	1	20	25
nutrient ovule		3	3							10	16
abnormal embryo sac								1		4	5
Total	12	22	29	12	20	8	4	7	7	54	175

Abbreviations: m. c., megasporocyte. All of embryo sac have egg apparatus but the number of polar nuclei and antipodals are variable; for example, a formula, 3+2+3, indicates an embryo state with three nuclei of egg apparatus, two polar nuclei, and three antipodals; X, uncertain number of antipodals.



Figs. 10–18. Megasporogenesis and megagametogenesis of *Euchresta japonica*. 10. Sporogenous cell at initiation of outer and inner integuments. 11. One large megasporocyte. 12. One elongated megasporocyte. 13–14. Two sections from the same ovule depicting linear tetrad. The cell nearest chalazal end becomes functional megaspore. Numbers 1, 2, 3 indicate degenerating cells. 15–16. Two sections from the same ovule depicting 4 nuclei after the second division. 17–18. Two sections from the same ovule depicting mature embryo sac. The flower size is 11 mm. 17. Egg apparatus. 18. Fused polar nuclei. Abbreviations: dm, degenerating megaspore; dn, degenerating nucellar tissue; eg, egg cell; fm, functional megaspore; fn, fused polar nuclei; ii, inner integument; mo, megasporocyte; nep, nucellar epidermis; oi, outer integument; sg, sporogenous cell; sy, synergids. Bars = 20 μm.

globular and strongly curved at the chalazal end, while that of *T. lupinoides* is elliptical. The nucellus of *E. japonica* is somewhat parallel to the ovary wall similar to that of other species, but its micropylar part is strongly curved toward the ovary wall. According to the classification of ovules by Bocquet and Bersier (1960), *E. japonica* is campylotropous; *T. lupinoides* is campylotropous to anatropous; *Sop. flavescens* and *M. amurensis* are amphitropous; *C. sikokiana*, *Sty. japonicum* and *Sop. tomentosa* are hemitropous.

Most of ovules curve toward the apex of the ovary, but in *Sty. japonicum* some ovules curve toward the base. Sometimes the inner integument and nucellar tissue elongate and protrude out of the outer integument. Even in these cases normal embryo sacs are observed.

Ovules which contain vessel elements with spiral thickenings are observed only in *E. japonica* and *M. amurensis.*

It is common in all species examined that the inner integument consists of two layers of cells. On the other hand, the number of cell layers of the outer integument differs among species. The outer integument consists of 2–3 layers in *C. sikokiana*, *Sty. japonicum*, *Sop. flavescens* and *M. amurensis*, of 4–5 layers in *Sop. tomentosa* and *T. lupinoides*, and of 7–10 layers in *E. japonica.* In *C. sikokiana* and *Sty. japonicum* the cell wall of the outermost layer of the outer integument has fibrous thickenings.

The micropylar end of the nucellus consists of 5 to 8 cell layers in *C. sikokiana*, *Sty. japonicum* and *M. amurensis*, while it is 2–4 cell layers in other species. *Sty. japonicum* has the thickest micropylar nucellus.

Megaspore tetrads are arranged in a linear pattern, in which the most chalazal megaspore is functional, in four species: *C. sikokiana*, *Sty. japonicum*, *T. lupinoides* and *E. japonica.* In *T. lupinoides*, however, T-shape pattern is also observed.

The size of the embryo sac correlates with that of the ovule (Fig. 19), that is, the larger embryo sac is included in the larger ovule. Starch grains are observed in the embryo sacs of *Sty. japonicum* and *M. amurensis*, which are frequently formed around the egg apparatus, polar nuclei and antipodals.

Antipodals are so ephemeral in *E. japonica* that they degenerate soon after completion of the embryo sac. In *C. sikokiana* they also degenerate at the mature stage. In other species, on the other hand, they still remain in the mature embryo sacs. Polar nuclei are fused into one layer nucleus before fertilization in *Sty. japonicum* and *M. amurensis*, while they are fused into one or in contact with each other in *C. sikokiana*, *T. lupinoides* and *E. japonica.* They are in contact in *Sop. tomentosa* and *Sop. flavescens.*

Discussion

Embryological features in *Euchresta japonica* are summarized as follows.

Anther tetrasporangiate; anther wall development the dicotyledonous type; tapetum glandular and uninucleate; cytokinesis in meiosis successive; mature pollen two celled at the time of shedding.

Ovule campylotropous, bitegmic and crassinucellate; outer integument seven to ten cell layers thick; vessel elements with spiral thickenings present; a single megaspore mother cell forms the linear tetrad of megaspores; chalazal megaspore developing into the Polygonum type embryo sac; three antipodals very ephemeral; starch grains absent in the embryo sac.

Few variations are known in anther anatomy, microsporogenesis and microgametogenesis in the subfamily Papilionoideae (e.g., Prakash 1987). In *E. japonica* anther wall development and microsporogenesis correspond to the general pattern reported so far in Papilionoideae (e.g., Prakash 1987).

Euchresta japonica has some peculiar ovu-

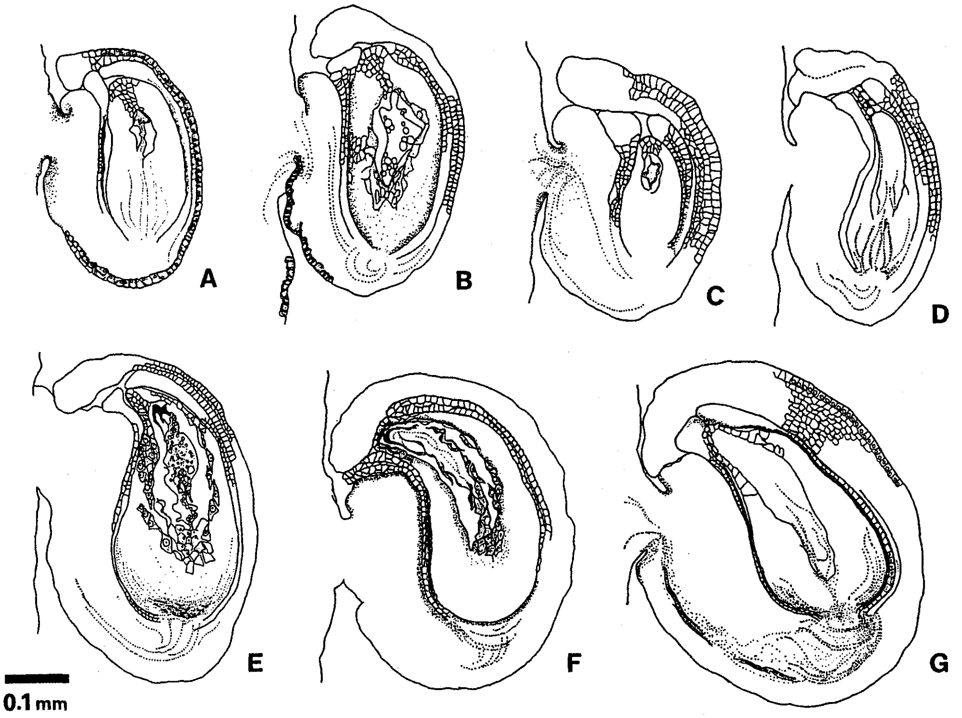


Fig. 19. Comparison of ovules among seven species examined. A. *Cladrastis sikokiana*. B. *Styphnolobium japonicum*. C. *Sophora tomentosa*. D. *Sophora flavescens*. E. *Maackia amurensis*. F. *Thermopsis lupinoides*. G. *Euchresta japonica*.

lar features in comparison with the other species examined in this study, that is, extremely curved ovules and a thick outer integument. The shape and size of ovules are closely related to the size of the ovary, number of ovules and the placentation (Eames 1961). One of the reasons that *E. japonica* has larger ovules is that a larger space is available for an ovule than other species because the ovary contains only one or two ovules (Table 2).

There are some differences in ovular morphology among the species examined. In *E. japonica* all of the mature ovules are strongly curved, that is, they are campylotropous. In *Sop. flavescens* and *T. lupinoides*, on the other hand, the ovules change from campylotropous like that of *E. japonica* at younger stages to amphitropous at the mature stage. Kostrikova (1979) also found a change in ovular shape in *Sop. flavescens*, that is, ana-campylotropous to nearly hemitropous. From these observations it may be considered that campylotropous in *E. japonica* may be a more primitive state than amphitropous in *Sop. flavescens* and *T. lupinoides*.

Rembert (1971) recognized linear, T-shaped, isobilateral and triad as the patterns of the megaspore tetrad in Leguminosae and suggested the phylogeny of the tetrad pattern in Leguminosae. In the subfamilies Mimosoideae and Caesalpinioideae and the tribe Sophoreae in Papilionoideae only the linear pattern is observed and this is thought to be the ancestral state. In the present work three species have only this pattern. *Euchresta japonica* has a common megaspore tetrad pattern with the more primitive group. The linear pattern is recently reported in *Dalbergia latifolia* of the

Table 2. Comparison of ovular characters among species examined

	Ovule number	Ovular width (mm)	Ovule curvature	Thickness of parietal cells	Thickness of outer integument	Outermost layer of outer integument	Nucellus at chalazal end	Vascular tissue
Cladrastis sikokiana	10–14	0.24	hemitropous	5 cells thick	2–3 cells thick	fibrous	clear	–
Styphnolobium japonicum	11–14	0.26	hemitropous	7–8 cells thick	2–3 cells thick	fibrous	degenerating	–
Sophora tomentosa	8–9	0.28	hemitropous	2–3 cells thick	4–5 cells thick	normal	clear	–
S. flavescens	10–13	0.25	amphitropous	3–4 cells thick	2–3 cells thick	normal	degenerating	–
Maackia amurensis	4–6	0.36	amphitropous	6 cells thick	2–3 cells thick	normal	degenerating	+
Euchresta japonica	1–2	0.46	campylotropous	2–3 cells thick	7–10 cells thick	normal	clear	+
Thermopsis lupinoides	13–15	0.38	campylotropous amphitropous	2–3 cells thick	4–5 cells thick	normal	degenerating	–

tribe Dalbergieae (Pandey et al. 1990), which is considered a primitive tribe of the subfamily Papilionoideae.

Embryo sac development is of the Polygonum type in almost all the genera of Leguminosae, but behaviour of the antipodals is variable in genera and sometimes even within a species (Prakash 1987). In geneal the antipodals remain until fertilization in Caesalpinioideae and Mimosoideae but are ephemeral in more advanced groups than Sophoreae of Papilionoideae (Prakash 1987) (Table 3). The present study reveals that the antipodals of *C. sikokiana* and *E. japonica* are ephemeral as in advanced groups of Papilionoideae, while those of other species examined remain late in the mature embryo sac, as in Caesalpinioideae and Mimosoideae. There are no reports on the behaviour of antipodals in Dalbergieae.

It has been suggested that *Euchresta* is closely related to *Sophora* (Ohashi and Sohma 1970) or *Maackia* (Chen et al. 1992). The present study revealed *E. japonica* has common features with *Sophora* in the thickness of pariental cells and the features of outermost layer of outer integument and nucellus at chalazal end, with *M. amurensis* in having small number of larger ovules with vessel elements, and with *C. sikokiana* in the feature of antipodals. However, because vessel elements were observed in many genera of Leguminosae (Prakash 1987), vascularization may not always mean close relationships.

Molecular systematic studies using the chloroplast-encoded *rbc*L gene showed that the tribe Sophoreae is polyphyletic (Doyle et al. 1997). *Cladrastis* and *Styphnolobium* are monophyletic and *Maackia* and *Sophora* are monophyletic. *Euchresta* is the sister taxon to the latter clade (Doyle et al. 1997). Our embryological data correspond to the results of the DNA analysis, that is, *Sty. japonica* is distinguishable from *M. amurensis*, *Sop.*

Table 3. Comparison of ovular characters among species in Leguminosae

Taxon	No. of ovules	Starch grains in embryo sac	Antipodals	Thickness of parietal cells	Vascular tissue	Authors
Mimosoideae			persist			Prakash (1987)
Caesalpinioideae			persist			Prakash (1987)
Papilionoideae						
Adesmia securigerifolia			persist		+	Primavera et al. (1994)
Cladrastis sikokiana	10–14	–	ephemeral	5 cells thick	–	present
Crotalaria juncea	10–16		ephemeral		–	Samal (1936)
Dalbergia latifolia	1–4	–		1–2 cells thick	+	Pandey et al. (1990)
Euchresta japonica	1–2	–	ephemeral	2–3 cells thick	+	present
Glottidium vesicarium		–	ephemeral	10–11 cells thick		Prakash and Herr (1979)
Glycine max	1–4	++	ephemeral	1–2 cells thick		Kennel and Horner (1985)
Indigofera cordifolia		+	ephemeral			Pllaiah (1995)
Lotus corniculatus	44–72	–	ephemeral?	1–2 cells thick		Hansen (1953)
Maackia amurensis	4–6	+	persist	6 cells thick	+	present
Medicago sativa	12–18	++	ephemeral			Martin (1914)
Melilotus alba	4–6	–	ephemeral	1–2 cells thick		Cooper (1933)
Melilotus officinalis	4–6	–	ephemeral	1–2 cells thick		Cooper (1933)
Psoralea corylifolia	1–2		persist		–	Joshi (1938)
Sophora flavescens	10–13	–	persist	3–4 cells thick	–	Kostrikova (1979), present
Sophora tomentosa	8–9	–	persist	2–3 cells thick	–	present
Styphnolobium japonicum	11–14		persist	7–8 cells thick	–	present
Thermopsis lupinoides	13–15	–	persist	2–3 cells thick	–	present
Trifolium pratense	1–3	–	ephemeral	2–3 cells thick		Martin (1914)
Vicia americana	12–18	+	ephemeral	1–2 cells thick		Martin (1914)
Wisteria floribunda	1–11	–	ephemeral	3–4 cells thick		Ohmiya and Ohashi (1993)

flavescens and *E. japonica* in the features of ovule and outer integument. There is no similarity in the ovular characters between *C. sikokiana* and *E. japonica* except for the ephemeral antipodals. If we could suppose the ephemeral antipodals common to *C. sikokiana* and *E. japonica* may be the result of parallel evolution, *Euchresta* may be an advanced group related to *Sophora* and *Maackia*.

We are grateful to the late Drs. Tsugiwo Yamanaka and Mitsuo Yamanaka who were Professors of Kochi University and have greatly contributed to flora and vegetation of Shikoku, for their kind and invaluable help for finding and collecting *Euchresta japonica* in Kochi Prefecture, Mr. K. Hayasaka (Kochi University), Dr. Y. Endo (Natural History Museum and Institute, Chiba), Dr. H. Iketani (Fruit Tree Research Station, Ministry of Agriculture, Forestry and Fisheries), Dr. H. Hoshi (National Forest Tree Breeding Center of Forestry Agency, Ministry of Agriculture, Forestry and Fisheries), Dr. Y. Iokawa (Jyoetsu University of Education), Mr. J. Yokoyama and Mr. T. Fukuda (Tohoku University), for collecting materials used in this study. We thank Prof. H. Tobe (Kyoto University) for his technical help, Dr. T. Kajita (Botanical Gardens, Graduate School of Science, University of Tokyo) for his suggestions based on DNA analysis on *Euchresta*, Mrs. Kazuko Goshima for her help in preparing our material for herbarium specimens and Mr. Kenichi Sato for cultivation of *Euchresta* plants in Experimental Gardens of our Biological Institute.

References

Bentham G. 1860. A synopsis of the Dalbergieae, a tribe of the Leguminosae. J. Linn. Soc. **9**: Suppl.: 1–134.

——— and Hooker J. D. 1865. Tribus Dalbergieae Leguminosae. Genera Plantarum **1**: 544–552. Reeve and Co., London.

Bocquet G. and Bersier J. D. 1960. La valeur systématique de l'ovule: Développements tératalogiques. Arch. Sci. (Geneva) **13**: 475–496.

Chen C., Sun H. and Mizuno M. 1992. On the genus *Euchresta* Benn. (Leguminosae) with "Wallece's line." Act. Phytotax. Sin. **30**: 43–56.

Cooper D. C. 1933. Macrosporogenesis and embryology of *Melilotus*. Bot. Gaz. **95**: 143–155.

Davis G. L. 1966. Systematic Embryology of Angiosperms. John Wiley, New York.

Doyle J. J., Doyle J. J., Ballenger J. A., Dickson E. E., Kajita T. and Ohashi H. 1997. A phylogeny of the chloroplast gene *rbc*L in the Leguminosae: Taxonomic correlations and insights into the evolution of nodulation. Amer. J. Bot. **84**: 541–554.

Eames A. J. 1961. Morphology of the Angiosperms. McGraw-Hill Book Co., New York.

Hansen H. W. 1953. Developmental morphology of *Lotus corniculatus*. Iowa State Coll. J. Sci. **27**: 563–600.

Herr J. M. Jr. 1984. Embryology and taxonomy. *In*: Johri B. M. (ed.), Embryology of Angiosperms. pp. 647–696. Springer-Verlag, Berlin.

Hutchinson J. 1964. Tribe Geoffroeeae. The Genera of Flowering Plants **1**: 390–393.

Joshi A. C. 1938. A note on the morphology of the gynoecium, ovule and embryo sac of *Psoralea corylifolia* L. J. Ind. Bot. Soc. **17**: 169–172.

Kennel J. C. and Horner H. T. 1985. Megasporogenesis and megagametogenesis in soybean *Glycine max*. Am. J. Bot. **72**: 1553–1564.

Kostrikova L. N. 1979. To the embriology of *Sophora flavescens* Ait. (Papilionaceae–Sophoreae). Vestn. Moskovsk. Univ., Ser 6, Biol. **34**: 26–31 (in Russian with English summary).

Maheshwari P. 1963. Embryology in relation to taxonomy. Vistas in Botany **4**: 55–97.

——— and Kapil R. N. 1966. Some Indian contributions to the embryology of angiosperms. Phytomorphology **16**: 239–291.

Martin J. N. 1914. Comparative morphology of some Leguminosae. Bot. Gaz. **58**: 154–167.

Ohashi H. 1973. Euchresteae, a new tribe of family Leguminosae. J. Jpn. Bot. **48**: 225–234.

——— and Sohma K. 1970. A revision of the genus *Euchresta* (Leguminosae). J. Fac. Sci. Univ. Tokyo, III (Bot.) **10**: 207–231.

Ohmiya T. and Ohashi H. 1993. Embryology of *Wisteria floribunda* (Willd.) DC. (Leguminosae) and the taxonomic position of the genus. J. Jpn. Bot. **68**: 326–333.

Palser B. F. 1975. The bases of angiosperm phylogeny: embryology. Ann. Missouri Bot. Gard. **62**: 621–646.

Pandey A. K., Jha S. S. and Jha A. 1990. Development and structure of seed in some Dalbergieae (Papilionoideae, Fabaceae). J. Ind. Bot. Soc. **69**: 107–113.

Pllaiah T. 1995. Embryology of *Indigofera* (Fabaceae).

Taiwania **40**: 391–402.
Polhill R. M. 1981. Tribe 30. Euchresteae (Nakai) Ohashi (1973). *In*: Polhill R. M. and Raven P. H. (eds.), Advances in Legume Systematics. Part **1**. Royal Botanic Gardens, Kew.
——— 1994. Classification of the Leguminosae. *In*: Bisby F. A., Buckingham J. and Harborne J. B. (eds.), Phytochemical Dictionary of the Leguminosae. pp. xxxv–lvii. Chapman and Hall, New York.
Prakash N. 1987. Embryology of Leguminosae. *In*: Stirton C. H. (ed.), Advances in Legume Systematics, Part 3, pp. 241–278. Royal Botanic Gardens, Kew.
——— and Herr J. M. Jr. 1979. Embryological study of *Glottidium vesicarium* through the use of clearing technique. Phytomorphology **29**: 71–77.
Primavera I., Stella M. and Rosario B. 1994. Seed ontogeny in *Adesmia securigerifolia* (Fabaceae-Adesmieae). Nord. J. Bot. **14**: 547–556.
Rembert D. H. Jr. 1971. Phylogenetic significance of megaspore tetrad patterns in Leguminales. Phytomorphology **21**: 1–9.
Sass J. E. 1958. Botanical Microtechnique. The Iowa State College Press. Ames, Iowa.
Samal K. K. 1936. The development of embryo sac and embryo in *Crotalaria juncea* L. J. Ind. Bot. Soc. **15**: 19–31.
Tobe H. 1989. The embryology of angiosperms: Its broad application to the systematic and evolutionary study. Bot. Mag. Tokyo **102**: 351–367.

大橋広好・田中和恵・根本智行：**マメ科ミヤマトベラの胚発生学的研究**

マメ科マメ亜科に属するミヤマトベラ属は，当初ツルサイカチ連Dalbergieaeに分類されていたが(Bentham 1860, 1865)，外部形態と花粉形態に基づいて，クララ属に類縁があると訂正された(Ohashi and Sohma 1970)．さらに，染色体とキノリジン・アルカロイドの比較研究により，独立の連を構成するものと考えられ，この1属よりなるミヤマトベラ連が設立された(Ohashi 1973)．その後，ミヤマトベラ属はイヌエンジュ属に類縁がある（Chen et al. 1992）とする説が発表された．さらに葉緑体DNAの*rbcL*分析に基づく分子系統学的な研究によると，この属はクララ属，イヌエンジュ属，センダイハギ連に近縁であることが示されている（Doyle et al. 1997）．そこで，ミヤマトベラ属の類縁関係を探るために，属あるいは連のレベルの分類群の系統関係推定に有効であると考えられる胚発生学的形質を調べた．これまで本属についてのこの分野での研究は全く発表されていない．本研究ではミヤマトベラの小胞子と大胞子の形成過程を観察し，形成様式を明らかにした．また，成熟した大胞子と胚嚢の形成過程を，類縁性が示唆されるグループと比較することによって，本属の分類学的位置についても考察した．

まず，ミヤマトベラの胚発生学的特徴は以下の通りであった．葯室は4，葯壁形成は双子葉型，タペート組織は1層分泌型で単核性，小胞子4分子は4面体型で成熟した花粉は2細胞であった．子房は1心皮からなり，胚珠数は多くは1個，湾生胚珠で珠皮は2枚，外珠皮は7–9層と厚い．珠心は厚層珠心で多くは1個の大胞子母細胞を生じるが，2個を生じることもある．大胞子4分子は1組を生じ，線型，胚嚢形成はタデ型で反足細胞は胚嚢形成が完了すると初期に消失してしまう．胚嚢は成熟に伴い珠心細胞を破壊し，合点側に管状に伸長する．極核は受精前に接触し，多くは融合する．

次に，ミヤマトベラ連との類縁性が指摘されるクララ連の中からクララ，イソフジ，エンジュ，イヌエンジュ，ユクノキ，センダイハギ連のセンダイハギを選定し，これらの種についても特に大胞子と胚嚢の形成過程を調べ，ミヤマトベラと比較した．胚珠の形態はミヤマトベラが最も湾曲している．外珠皮はミヤマトベラでは7–10層，他の種では2–5層であった．大胞子4分子の形態はミヤマトベラ，ユクノキ，センダイハギともに線型で胚嚢形成はタデ型である．また，ミヤマトベラでは胚嚢が完成すると初期の段階で反足細胞は消失してしまうが，ユクノキを除く他の種では全て成熟するまで残っていた．

以上の結果から，ミヤマトベラの胚発生学的特徴はクララ連ともセンダイハギ連とも異なっている．しかし，反足細胞が短命である特徴はマメ科植物では進化したと推定されるグループで多く見られることとクララ連のユクノキでも見られたことで，ミヤマトベラ連も進化的な特徴をもつと考えられる．クララ連は分子系統的にも多系統であることが示唆されており（Doyle et al. 1997），クララ連とミヤマトベラ連とのより具体的な系統関係についてさらに研究が必要である．

（東北大学大学院理学研究科生物学教室）